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Elucidating the ecology of bucephalid parasites using a mutation scanning approach☆

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Abstract

Nucleotide variation in a portion of the mitochondrial cytochrome *c* oxidase subunit1 (*cox*1) gene from asexual stages of bucephalids of southern Australian scallops (*Chlamys asperrima*, *Chlamys bifrons* and *Pecten fumatus*) was investigated using a mutation scanning–sequencing approach. Single-strand conformation polymorphism (SSCP) analysis revealed three main profile types (A, B and C) for parasites isolated from scallops. Sequence analysis revealed that samples represented by profiles B and C had a high degree (97.3%) of sequence similarity, whereas they were ~21% different in sequence from those represented by profile A. These findings suggested that at least two types or species (represented by profile A, or profile B or C) of bucephalid infect scallops, of which both were detected in South Australia, while only one was found in Victoria. The prevalence of bucephalids (and their SSCP haplotypes) appeared to differ among the three species of scallop in South Australia as well as between the two scallop species in Victoria, indicating a degree of host specificity. Adult bucephalids were collected from Eastern Australian Salmon (*Arripis trutta*), in an attempt to match them with the asexual stages from the scallop hosts. Neither of the two taxa of adult bucephalid (*Telorhynchus arripidis* and an un-named *Telorhynchus* species) shared SSCP profiles with the bucephalids from scallops, but were genetically similar, suggesting that the asexual stages from scallops may represent the genus *Telorhynchus*. This study, which assessed nucleotide sequence variation in a portion of the mitochondrial *cox*1 gene for bucephalids found in scallops and arripid fish, illustrates the usefulness of the mutation scanning approach to elucidate complex life-cycles of marine parasites.

Keywords: Scallop; Digenea; Bucephalid; Identification; Host specificity; Genetic variation; Single-strand conformation polymorphism analysis; Mitochondrial DNA

1. Introduction

Digeneans are important ecological indicators, their transmission pathways providing extensive information on host biology, trophic interactions and food-web dynamics [1]. However, digenean life-cycles are complex and difficult to elucidate, because they usually involve several different host species and dispersive stages [1]. Recently, molecular techniques have proved valuable in enabling the specific identification of parasites where morphological features are of limited or no value [2]. Consequently, complex parasite life-cycles may be unravelled by genetically matching life stages from different host species.

Digeneans of the family Bucephalidae parasitise many species of mollusc, including commercially important bivalve molluscs, such as mussels, clams, oysters, cockles and scallops [3]. They cause reproductive failure of their intermediate mollusc host by progressively replacing gonad tissue with asexual life stages, known as sporocysts [3]. Millions of two-tailed sexual cercariae are produced within the sporocysts, which escape from the mollusc into water

☆ Nucleotide sequence data reported in this paper are available in the EMBL, GenBank™ and DDJB databases under accession numbers AY504854–AY504864.

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and swim until they find their second intermediate (teleost) host and penetrate its skin [4]. To complete the cycle, the second intermediate host is eaten by a definitive teleost host, in which the ingested bucephalid attaches to the intestine of the new host, develops into an adult, reproduces sexually and releases eggs in the faeces. [4]. Eggs hatch to release motile miracidia, which then infect mollusc intermediate hosts [3].

Scallops are well-known hosts of bucephalids. In Australia, infections have been detected in populations of *Pecten fumatus* and *Chlamys asperrima* from New South Wales [4], *P. fumatus* from Victoria [6], *P. fumatus* and *C. asperrima* from Bass Strait (West, personal communication), and *P. fumatus*, *C. asperrima* and *Chlamys bifrons* from South Australia (Styan and Keough, unpublished data). However, the bucephalids which infect these scallops have yet to be identified to genus or species, and their second intermediate and definitive teleost fish hosts are presently unknown.

Adult bucephalids have been recorded and described for a number of species of southern Australian fish [7]. Of known definitive hosts, Eastern Australian Salmon (*Arripis trutta*) is probably the most common and widespread species, with migratory populations found along the coasts of New South Wales, Tasmania and Victoria [8]. *A. trutta* is a known host for at least four species of bucephalid parasite, including *Telorhynchoides longicollis*, and *Telorhynchus arripidis*, *T. kahawai* and *T. peacheyii* [9]. A closely related species, the Western Australian Salmon (*Arripis truttaceus*), could also be a potential definitive host for bucephalids and occurs in large numbers in South Australia and Western Australia. The ranges of the two species of *Arripis* overlap in Victorian and Tasmanian waters [8]. Although arripids are common in locations where scallops are also found, it is not known whether bucephalids of scallops represent the asexual stages of one or more of those found in *A. trutta* or *A. truttaceus*.

It is also not known whether only one species of bucephalid infects different species of southern Australian scallop. The prevalence of bucephalid intramolluscan stages has been found to differ significantly between different host species occurring in sympatry, and can change independently through time ([5]; Styan and Keough, unpublished data). One explanation for these observations might be that separate species of bucephalid infect different scallop species. A key problem in resolving this, and addressing questions about the identity of second intermediate and definitive hosts, is that species of bucephalids are difficult to distinguish based on the morphology of their asexual stages [9].

Complex parasite life-cycles may be unravelled by genetically matching life stages from different host species, an approach employed herein. The aims of this study were (a) to investigate genetic variation within and among asexual parasite stages from three sympatric scallop species, in order to determine whether one or more bucephalid species infect different host species and/or are found in different locations and (b) to test the genetic match or relatedness between the unidentified asexual stages of bucephalids from scallops with known adult stages from a possible primary host, *A. trutta*. To do this, PCR-based single-strand conformation polymorphism analysis (PCR-SSCP) [10] followed by selective DNA sequencing was employed to characterise nucleotide variation in the mitochondrial cytochrome *c* oxidase subunit 1 gene (*cox*1) within and among parasite isolates. Such a mutation scanning approach has been shown previously to be effective for studying population genetic structures [11–14].

2. Materials and methods

2.1. Collection and processing of trematode parasites from scallops and fish

Sampling locations are shown in Fig. 1. The scallops, *C. asperrima*, *C. bifrons* and *P. fumatus*, were collected by divers in South Australia during October 2000. All three species were collected from under Edithburgh Jetty, Port Giles Jetty and Klein Point Jetty in Gulf St Vincent. *C. bifrons* were collected from under Port Hughes Jetty in the Spencer Gulf. *P. fumatus* was rare in all locations in South Australia. In Port Phillip Bay, *P. fumatus* was collected off-shore from Dromana, Mt Martha, Rye, McCrae and Portarlington by divers in October and November 2000; some were also collected from a trawler with dredges taken from Clifton Springs and Werribee South. *C. asperrima* was collected from Rye Pier, Portsea Reef and Point Franklin, Port Phillip Bay in November and December 2000. No *C. bifrons* were detected during any collections in Port Phillip Bay. For analyses, scallops were treated as coming from either Spencer Gulf, Gulf St Vincent (both South Australia) or Port Phillip Bay (Victoria). Only adult scallops (those large enough to have mature gonads in uninfected individuals) were collected throughout; this corresponded to animals with shell widths greater than ~45 mm (*C. asperrima*) and ~55 mm (*P. fumatus* and *C. bifrons*). Parasitised gonads were always red in colour and easily distinguished from healthy gonads [6]. For the isolation of DNA, sections (~3 mm^3) from the end of infected gonads were excised. These were each squashed with a clean slide and then washed with physiological saline. A volume of ~200 μl of each gonad/saline suspension was pipetted into an Eppendorf tube. For the first parasitised samples, the suspensions were centrifuged and the sediment examined microscopically for sporocysts. To obtain parasite control samples, cercariae were shed from gonad tissue into H_2O. The morphology of these cercariae was consistent with the family Bucephalidae.

Specimens of *A. trutta* were caught on lures at flood tide, Point Nepean, Port Phillip Bay in February 2001. The fish were distinguished from *A. truttaceus* by the number of gill rakers on the first gill bar [8]. The digestive tracts were removed, opened and shaken vigorously in physiological saline. The settled contents were sorted under a dissecting

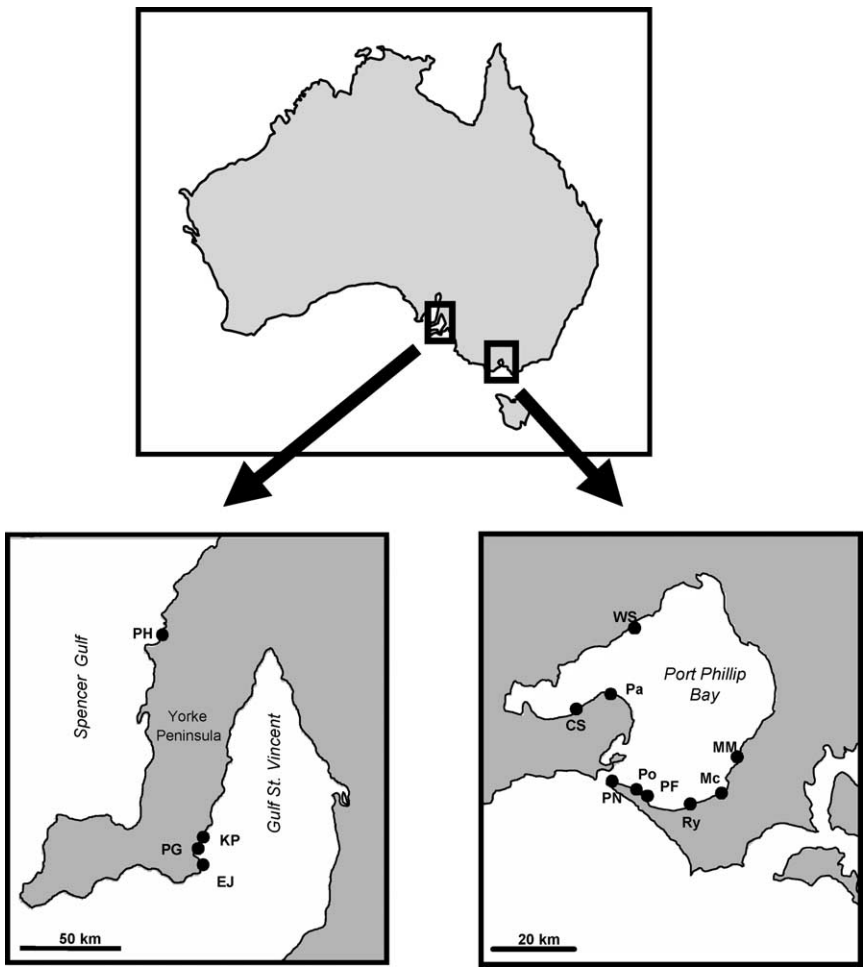

Fig. 1. Locations where potential hosts to bucephalids were collected across southern Australia. In Spencer Gulf, South Australia, Port Hughes (PH); in Gulf St Vincent, South Australia, Klein Point (KP), Port Giles (PG), Edithburgh Jetty (EJ); in Port Phillip Bay, Victoria, Port Nepean (PN), Porstsea (Po), Point Franklin (PF), Rye (Ry), McCrae (Mc), Mount Martha (MM), Werribee South (WS), Clifton Springs (CS) and Portarlington (Pa).

microscope, and adult trematodes were aspirated with a pipette. For each fish, some trematodes were fixed in 70% ethanol for 24 h, whereas others were transferred to Eppendorf tubes for freezing and subsequent DNA isolation and molecular analysis. Fixed trematodes were placed in distilled H_2O before being stained in Celestine Blue solution for 30 min, then destained in 1% HCl in 70% ethanol. Destaining was inhibited with 1% ammonia in 70% ethanol. The trematodes were dehydrated in alcohol (70–90–95–100%) before being cleared in methyl salicylate for 30 min and mounted on a glass slide with Canada balsam. Bucephalids were identified under a compound microscope using a key to Australian trematode families (provided by Cribb, unpublished data). Two bucephalids were identified, namely *T. arripidis* and an un-named species of *Telorhynchus*, according to the descriptions of Crowcroft [15] and Lebedev [16].

2.2. Isolation of genomic DNA and enzymatic amplification

Total genomic DNA was isolated from uninfected host tissue, infected scallop tissue, from cercariae or from individual bucephalid adults using sodium dodecylsulphate/proteinase K treatment [17], followed by purification over Wizard™ Clean-Up columns (Promega) and elution in 50 μl H_2O. DNA was purified in the same way from uninfected host tissues.

Part of the *cox* 1 gene (p*cox* 1; ~340 bp), was amplified by PCR from 10–20 ng of genomic DNA using primers JB3 (5′-TTTTTTGGGCATCCTGAGGTTTAT-3′; forward) and JB4.5 (5′-TAAAGAAAGAACATAATGAAAATG-3′; reverse) [2] designed to *cox*1 regions of *Fasciola hepatica* [18]. PCR reactions (50 μl) were performed in 10 mM Tris–HCl pH 8.4, 50 mM KCl, 3.5 mM $MgCl_2$, 250 mM of each dNTP, 50 pmol of each primer and 1U *Taq* polymerase (Promega) using the following conditions: 94 °C for 5 min (initial denaturation), followed by 30 cycles of 94 °C for 30 s (denaturation), 55 °C for 30 s (annealing), 72 °C for 30 s (extension), followed by a final extension at 72 °C for 5 min. Samples without parasite or with host genomic DNA were also included in amplification runs as 'negative' controls. An aliquot (4 μl) of each PCR product was examined by agarose gel electrophoresis [19].

2.3. Electrophoretic analysis of amplicons, sequencing and genetic analyses

The method of SSCP, described previously by Zhu and Gasser [20], was used to screen the p*cox*1 region for sequence variation within and among amplicons. In brief, 10 μl of each radiolabelled amplicon were mixed with an equal volume of loading buffer (10 mM NaOH, 95% formamide, 0.05% bromophenol blue and 0.05% xylene cyanole, Promega). After denaturation at 94 °C for 5 min and subsequent snap cooling on a freeze-block (−20 °C), 3 μl of each sample was subjected to electrophoresis (20 W for 6 h at 20 °C) in a 0.4 mm-thick mutation detection enhancement gel matrix (FMC BioProducts, USA). After electrophoresis, gels were dried on to blotting paper and subjected to autoradiography for 24 h.

Selected amplicons representing each of the main p*cox* 1 SSCP profiles were purified over mini-spin columns (Wizard™ PCR-Prep, Promega) and used as templates for subsequent automated sequencing (BigDye chemistry v.1; in a PE Applied Biosystems 377 sequencer) using primers JB3 and JB4.5 in separate reactions. Amino acid sequences were deduced from each nucleotide sequence using the program MacVector™ 4.1.4 (employing the invertebrate setting), in order to rule out the amplification and subsequent sequencing of 'pseudogenes' [21]. Nucleotide gene sequences were then aligned manually, and pairwise comparisons of sequence differences (D) were made using the formula $D = 1 - (M/L)$, where M is the number of alignment positions at which the two sequences have a base in common, and L is the total number of alignment positions over which the two sequences were compared [22]. A phenogram was constructed from these data using the unweighted pair group method using arithmetic averages (UPGMA) [23].

3. Results

3.1. Prevalence of bucephalids in scallops and in A. trutta

Bucephalids were much more prevalent in *P. fumatus* than in either of the *Chlamys* species in South Australia, and were only present in *P. fumatus* in Victoria (Table 1). Two species of adult bucephalid were collected from 14 *A. trutta*. One was identified as *T. arripidis* ($n = 17$), and the other represented an un-named *Telorhynchus* sp. ($n = 8$). *T. arripidis* and *Telorhynchus* sp. were characterised by the presence of a rhynchus at the anterior extremity, where the oral sucker is usually found. The gut opened through a mouth in the middle of the body. *T. arripidis* had 18 anterior spines, whereas *Telorhynchus* sp. had 13. *T arripidis* was found in six fish, while *Telorhynchus* sp. was found in five. Only one fish had both types of parasite, whereas four had neither.

3.2. SSCP analysis and selective sequencing

Amplicons were produced from all samples containing parasites, and the cercarial control samples amplified consistently. Autoradiographic exposure of agarose gels containing the resolved amplicons confirmed their specificity and the PCR conditions, in that each amplicon appeared as a single, discrete band. No bands were detected in the no-DNA control and host DNA samples, and no size variation was detectable among the amplicons on agarose gels.

SSCP analysis of all amplicons ($n = 76$) showed five main profiles, each consisting of four to five bands. These profiles were labelled A, B, C, D and E, in the order in which they were discovered. Fig. 2 shows a representative SSCP gel with all of the profiles representing bucephalids from all three scallop species examined from all collection sites. This SSCP gel also included a negative control from *C. bifrons*, tissue (no band), a parasite control (cercarial DNA) from *P. fumatus* and the sample representing *T. arripidis*.

SSCP analysis revealed distinct profile-groupings, according to the scallop hosts. The frequencies of profiles A, B and C in the scallops are shown in Table 2. In South Australia, profile A was detected only in *C. asperrima* and *C. bifrons* whereas profile B was detected in all three scallop species, although only once in *C. asperrima*. Profile C was present exclusively in *C. bifrons* and *P. fumatus*. Profiles B and C were equally prevalent in *P. fumatus* in Victoria, but absent from *C. asperrima* there. Profile A was not detected

Table 1
Prevalence of parasitism due to bucephalids in scallops and adult bucephalids in *Arripis trutta* in South Australia (SA) and Victoria (VIC)

Location	Host species	Parasite	Total no. of hosts infected	Total no. of hosts sampled	Percentage of hosts infected
Gulf St Vincent, SA	*Chlamys asperrima*	Bucephalid	14	371	3.7% (*2.1, 6.3*)
	Chlamys bifrons	Bucephalid	15	402	3.7% (*2.1, 6.1*)
	Pecten fumatus	Bucephalid	6	11	54.5% (*23.4, 83.3*)
Spencer Gulf, SA	*Chlamys bifrons*	Bucephalid	3	70	4.3% (*0.9, 7.9*)
Port Phillip Bay, VIC	*Chlamys asperrima*	Bucephalid	0	234	0 (*0, 1.6*)
	Pecten fumatus	Bucephalid	32[a]	524[a]	5.5% (*3.7, 7.9*)[a]
	Arripis trutta	*Telorhynchus arripidis*	6	14	42.9% (*17.7, 71.1*)
		Telorynchus sp.	5	14	35.7% (*12.8, 64.9*)

Italicised values in brackets are lower, upper 95% confidence estimates of prevalence.
[a] Only scallops collected by divers in Port Phillip Bay were included.

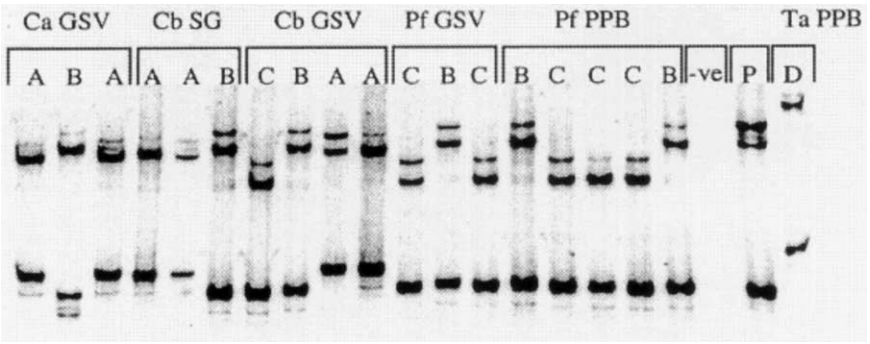

Fig. 2. Single-strand conformation polymorphism gel showing banding patterns associated with a portion (p*cox* 1) of the mitochondrial cytochrome *c* oxidase subunit 1 gene. Representative profiles (A–D) of asexual intramolluscan stages and adult bucephalids are labelled above the corresponding lane. Profiles are boxed together, indicating parasite host species, (*Chlamys biforns* (Cb), *Chiamys asperrima* (Ca), *Pecten fumatus* (Pf), *Telorhynchus arripidis* (Ta)) and location in southern Australia (Gulf St Vincent (GSV), Spencer Gulf (SG) and Port Phillip Bay (PPB)). A negative control, (tissue from uninfected *C. bifrons*) and a parasite control, P, (shed cercariae from *P. fumatus*) are shown.

in scallops collected from Victoria. Profiles D and E represented *T. arripidis* and *Teloryhnchus* sp., respectively.

Ten samples representing all of the different SSCP profiles detected (including multiple samples for some profiles) were subjected to sequencing, and nine different *cox*l sequence types (haplotypes) were defined (see Table 3). Pairwise comparisons of these haplotypes (for an alignment length of 336 bp; no indels) revealed low-level sequence variation among samples representing each profile A, B and C (Table 3). Nucleotide variation of up to 1.2, 0.6 and 1.5% was detected among samples representing profiles A, B and C, respectively. All samples represented by profile A possessed the same predicted COX1 peptide sequence (for an alignment length of 112 amino acids). Also, all samples represented by profiles B and C had an identical COX1 sequence.

There was a high level of nucleotide sequence difference between samples with profile A and those with profile B (19.6–21.4%), as well as between samples with A and those with profile C (19.6–22.3%). In contrast, the level of sequence difference between samples with profiles B and those with profile C was substantially lower (1.2–2.7%). Nine amino acid differences (8%) were detected in the COX1 sequence between samples with profile A compared with those with profile B or C.

Samples with profiles A and B or C were genetically more similar to one another than to *T. arripidis*, which differed by up to 32% in nucleotide sequence. The un-named *Telorhynchus* sp. was the most distinct genetically, differing from the molluscan bucephalids by up to ~40%, and being distinct from *T. arripidis* (~43%).

3.3. Genetic relationships

A phenogram (Fig. 3), constructed using the p*cox*l nucleotide sequence data, displays the genetic difference among all bucephalid samples, including asexual intramolluscan stages and the known adult bucephalid, *T. arripidis*. The p*cox*1 haplotypes representing profile B clustered with those representing profile C, thus being more similar in sequence to one another than to the haplotypes representing profile A. *T. arripidis* grouped external to the two molluscan bucephalid clusters.

4. Discussion

Mutation scanning analysis, followed by sequencing, suggested that there are at least two different types of bucephalid parasite infecting southern Australian scallops.

Table 2
Frequency of p*cox* 1 SSCP profiles for bucephalids from scallops from South Australia (SA) and Victoria (VIC)

	Gulf St Vincent, SA			Spencer Gulf, SA	Port Phillip Bay, VIC	
	Chlamys bifrons	*Chlamys asperrima*	*Pecten fumatus*	*Chlamys bifrons*	*Chlamys asperrima*	*Pecten fumatus*
SSCP profile						
A	7	13	–	2	–	–
B	3	1	2	1	–	16
C	5	–	4	–	–	16
Total no. of hosts infected	15	14	6	3	0	32

The total number of each SSCP profile found in a collection of scallops is shown. A dash indicates that a particular profile was not detected.

Table 3
Pairwise comparison of sequence differences (%) in a portion of the mitochondrial cyctochrome *c* oxidase subunit 1 gene (336 bp) among samples representing bucephalids

	DTaPPB	BPfGSV	CPfGSV	CCbGSV	BCbGSV	ACbSG	ACbGSV	ACaGSV	CPfPPB	BPfPPB
DTaPPB	*	*	*	*	*	*	*	*	*	*
BPfGSV	28.9	*	*	*	*	*	*	*	*	*
CPfGSV	30.7	2.4	*	*	*	*	*	*	*	*
CCbGSV	31.5	1.5	1.5	*	*	*	*	*	*	*
BCbGSV	30.0	0.0	2.4	1.2		*	*	*	*	*
ACbSG	28.0	21.1	22.3	21.1	21.1	*	*	*	*	*
ACbGSV	27.6	19.6	21.4	20.8	20.0	0.3	*	*	*	*
ACaGSV	28.0	20.4	21.7	20.8	20.8	1.2	1.2	*	*	*
CPfPPB	31.2	1.2	1.2	0.3	1.2	21.4	20.4	19.6	*	*
BPfPPB	30.6	0.6	2.7	1.5	0.3	21.1	21.1	21.4	1.5	*

Codes of individual samples relate to SSCP profile (A–D), host species (*Chlamys bifrons* (Cb), *Chlamys asperrima* (Ca), *Pecten fumatus* (Pf), *Telorhynchus arripidis* (Ta)), and location (Gulf St Vincent (GSV), Spencer Gulf (SG), and Port Phillip Bay (PPB) in southern Australia); *, no value.

The samples representing SSCP profile B and those representing profile C were very similar in nucleotide sequence (97.3%), but they were both distinctly different from those representing profile A. Indeed, 66–75 nucleotide differences (~22%) between samples representing profile A and those representing profiles B/C provided support for the proposal that they are different types or species of parasite. This was reinforced by the considerable difference (8%) in the predicted COX1 amino acid sequence. Previous studies assessing *cox*l sequence divergence in trematodes and cestodes have accepted even lower levels of divergence (e.g. 5–14%; mean: 9%) to discriminate species [24,25].

The sequence variation between samples representing each profile B and profile C was low (<2.7%). Mitochondrial DNA sequence divergence detected within populations of trematode species is usually low, and a 2.7% sequence difference between samples representing profile B and profile C is consistent with population (or intraspecific) variation determined in other studies for a diverse range of taxa (except nematodes) [26,27]. Also, there was no amino acid sequence difference between samples representing profile B and those representing profile C. Hence, the low level of nucleotide variation recorded among representatives of profiles B and C suggested that they represented the same species. Interestingly, there was no obvious geographical separation of the *cox*l haplotypes among populations from South Australia and Victoria.

Finding at least two types of intramolluscan bucephalid provides a simple explanation for the substantial differences in the overall prevalence and dynamics of parasite-induced castration among sympatric scallop host species. Major differences in bucephalid prevalence between *P. fumatus* and *C. asperrima* were observed during this study in South Australia and Victoria, and have been noted previously in New South Wales [4]. The differences may relate to distinct types of bucephalid infecting each host, such as those represented by profile A in *C. asperrima* (which was absent from Victoria) and those represented by profile B or C in *P. fumatus*. The difference in parasite prevalence between *P. fumatus* and *C. bifrons*, however, cannot be so simply explained, because these hosts shared bucephalid types.

Possible explanations for variation in bucephalid exposure to suitable hosts include abiotic factors, such as temperature, salinity and water currents, and physiological factors, such as cues used by invasive larval stages. One contributing factor to the prevalence of bucephalids observed here may be differences in the ecology of host species; *P. fumatus* sit flush in the sediment, *C. bifrons* sit on top of the sediment and *C. asperrima* bysally attach to surfaces in the water column. These different positions may thus relate to different levels of exposure to larval bucephalids.

The intramolluscan bucephalids represented by SSCP profiles A and B/C exhibit different patterns of prevalence within their scallop hosts. In sympatric populations of scallops in Gulf St Vincent, the type of bucephalid with profile A was most common in *C. asperrima* (3.5%) but was also present in *C. bifrons*, although at a lower prevalence (1.7%). This type was not found in *P. fumatus*. Profile B or

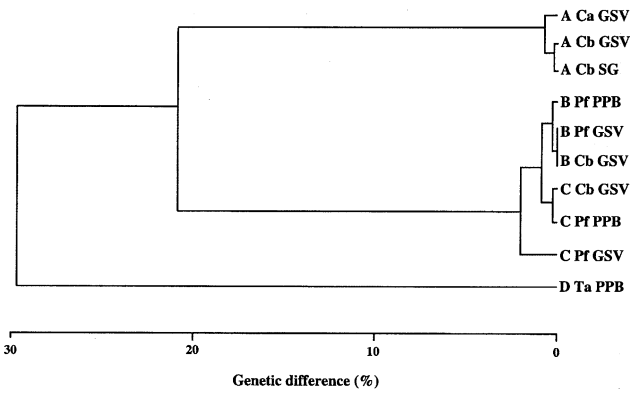

Fig. 3. UPGMA phenogram depicting genetic (nucleotide) difference in a portion of the mitochondrial cytochrome *c* oxidase subunit 1 region (336 bp) between asexual intramolluscan stages in scallops and *Telorhynchus arripidis*. The codes of individual samples relate to SSCP profile (A–D), host species (*C. bifrons* (Cb), *C. asperrima* (Ca), *P. fumatus* (Pf)), and location in southern Australia (Gulf St Vincent (GSV), Spencer Gulf (SG) and Port Phillip Bay (PPB)).

C was represented in all three scallop populations, but the prevalence varied markedly, from being very common in *P. fumatus* (54.5%), to being uncommon in *C. bifrons* (2%) and very rare in *C. asperrima* (0.3%). The reason for differences in prevalence is unclear. It appears that bucephalid-scallop interactions are host-specific and involve active mechanisms which manifest as differences in prevalence among scallop species in sympatry. Future work might determine whether parasitism is associated with behavioural adaptations of the infecting parasite larvae, such as larval choice, or whether there is differential resistance by scallops to infection.

The adult bucephalids examined in this study, *T. arripidis* and *Telorhynchus* sp., were not genetically matched to either of the bucephalids from scallops, and thus probably parasitise other first intermediate mollusc hosts. However, the level of nucleotide sequence difference between samples representing either profile A, B or C and *T. arripidis* was less than that between *T. arripidis* and *Telorhynchus* sp. Assuming that *Telorhynchus* is monophyletic and that the level of sequence divergence among taxa should reflect taxonomic relatedness, this would suggest that the two intramolluscan bucephalid types also represent *Telorhychus*, providing clues as to their specific identity and the fish hosts in which their respective adults might be found.

Telorhynchus has only been recorded from *A. trutta* in southern Australia [6]. If the asexual bucephalid stages in scallops represent *Telorhynchus*, their adults may also be found in *A. trutta*. The two bucephalids (*T. arripidis* and an undescribed *Telorhynchus* sp.) found in *A. trutta* can now, through the present study, be discounted as the adult parasites infecting scallops. However, future work might find a match with one or two of the remaining three named species of adult *Telorhynchus* which were not found in *A. trutta*. Given that a previously unidentified taxon of *Telorhynchus* was collected from 14 fish in this study, another possibility is that the adults of the species found in scallops may represent yet another undescribed species in *A. trutta*.

Other fish may also act as definitive hosts for *Telorhynchus*, and the genus has been recorded in other fish families in a number of waters [28,29]. That *Telorhynchus* has been recorded only from *A. trutta* in southern Australia may simply reflect that other potential fish hosts have not been sufficiently examined. Other members of the Arripidae in southern Australia might also act as definitive hosts for *Telorhynchus*, even though they have not yet been recorded. The eastern Australian Salmon, *A. truttaceus*, and the Tommy Rough, *A. georgiana*, are morphologically and ecologically similar to *A. trutta* [30]. Both species have distributional ranges which overlap with scallops infected with bucephalids and presumably eat similar, potential second intermediate host fish to *A. trutta*.

Identification of species in different hosts and at different life stages can be particularly difficult for marine parasites with complex life-cycles, yet it has major implications for studying the ecology and transmission patterns of parasites and for understanding the evolution of host–parasite relationships. The present study has demonstrated that such limitations can be overcome by using a molecular genetic approach, employing genetic markers in the *cox*l gene of mitochondrial DNA. This provides unique prospects for determining the life-cycles and ecology of these parasites in the near future.

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References

[1] Marcogliese DJ, Cone DK. Food webs: a plea for parasites. Trends Ecol Evol 1997;12:320–3.
[2] McManus DP, Bowles J. Molecular genetic approaches to parasite identification: their value in diagnostic parasitology and systematics. Int J Parasitol 1996;26:687–704.
[3] Cheng TC. Marine molluscs as hosts for symbioses with a review of known parasites of commercially important species. In: Russel FS, editor. Advances in marine biology. London: Academic Press; 1967.
[4] Williams H, Jones A. Parasitic worms of fish. London: Taylor and Francis; 1994.
[5] Heasman MP, O'Connor WA, Frazer AW. Digenean (Bucephalidae) infections in commercial scallops, *Pecten fumatus* (Reeve), and doughboy scallops, *Chlamys* (*Mimachlamys*) *asperrima* (Lamarck), in Jervis Bay, New South Wales. J Fish Dis 1996;19:33–9.
[6] Sanders MJ. Parasitic castration of the scallop *Pecten alba* (Tate) by a bucephalid trematode. Nature 1966;15:307–8.
[7] Beumer JP, Ashburner LD, Burbury ME, Jettè E, Latham DJ. A checklist of the parasites of fishes from Australia and its adjacent Antarctic territories. London: Commonwealth Agricultural Bureaux, Farnham Royal; 1983. p. 8–56.
[8] May JL, Maxwell JGH. Field guide to trawl fish from temperate waters of Australia. CSIRO Division of Fisheries Research, Melbourne, Australia: National Library of Australia Cataloguing-in-Publication Entry; 1986.
[9] Taskinen J, Valtonen ET, Gibson DI. Studies on bucephalid digeneans parasitizing mollusks and fishes in Finland I. Ecological data and experimental studies. Syst Parasitol 1991;19:1–94.
[10] Orita M, Suzuki Y, Sekiya T, Hayashi K. Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. Genomics 1989;5:874–9.
[11] Sunnucks P, Wilson ACC, Beheregaray LB, Zenger K, French J, Taylor AC. SSCP is not so difficult: the application and utility of single-stranded conformation polymorphism in evolutionary biology and molecular ecology. Mol Ecol 2000;9:1699–710.
[12] Zhu XQ, Beveridge I, Berger L, Barton D, Gasser RB. Single-strand conformation polymorphism-based analysis reveals genetic variation within *Spirometra erinacei* (Cestoda: Pseudophyllidea) from Australia. Mol Cell Probes 2002;16:159–65.
[13] de Gruijter JM, Polderman AM, Zhu XQ, Gasser RB. Screening for haplotypic variability within *Oesophagostomum bifurcum*

(Nematoda) employing a single-strand conformation polymorphism approach. Mol Cell Probes 2002;16:183–8.

[14] Hu M, Chilton NB, Gasser RB. The mitochondrial genomics of parasitic nematodes of socio-economic importance: recent progress, and implications for population genetics and systematics studies. Adv Parasitol 2004;56:133–212.

[15] Crowcroft PW. The anatomy of two new digenetic trematodes from Tasmanian food fishes. Proc Linn Soc New South Wales 1947;71: 108–18.

[16] Lebedev BI. Trematodes of the family Bucephalidae (Poche, 1907) of a commercial marine fish of New Zealand and Australia. Parazitologiya 1968;4:156–67. [in Russian].

[17] Gasser RB, Chilton NB, Hoste H, Mallet S, Beveridge I. Lack of intraspecific variation in the second internal transcribed spacer (ITS-2) of *Trichostrongylus colubriformis* ribosomal DNA. Int J Parasitol 1993;23:1069–71.

[18] Garey JR, Wolstenholme DR. Platyhelminth mitochondrial DNA: evidence for early evolutionary origin of a tRNA(serAGN) that contains a dihydrouridine arm replacement loop, and of serine-specifying AGA and AGG codons. J Mol Evol 1989;28:374–87.

[19] Sambrook J, Fritsch EF, Maniatis T. Molecular Cloning: A Laboratory Manual. New York: Cold Spring Harbor Laboratory Press; 1989.

[20] Zhu XQ, Gasser RB. Single-strand conformation polymorphism (SSCP)-based mutation scanning approaches to fingerprint sequence variation in ribosomal DNA of ascaridoid nematodes. Electrophoresis 1998;19:1336–73.

[21] Zhang DX, Hewitt GM. Nuclear integrations: challenges for mitochondrial DNA markers. Tree 1996;11:247–51.

[22] Chilton NB, Gasser RB, Beveridge I. Difference in a ribosomal DNA sequence of morphologically indistinguishable species within the *Hypodontus macropi* complex (Nematoda: Strongylida). Int J Parasitol 1995;25:647–51.

[23] Sneath PHA, Sokal RR. Numerical Taxonomy: The Principles and Prac-tice of Numerical Classification. San Francisco: WH Freeman and Co; 1973.

[24] Morgan JAT, Blair D. Relative merits of nuclear ribosomal internal transcribed spacers and mitochondrial CO1 and ND1 genes for distinguishing among *Echinostoma* species (Trematoda). Parasitology 1998;116:289–97.

[25] Okamoto M, Bessho Y, Kamiya M, Kurosawa T, Horii T. Phylogenetic relationships within *Taenia taeniaeformis* variants and other taeniid cestodes inferred from the nucleotide sequence of the cytochrome *c* oxidase subunit 1 gene. Parasitol Res 1995;81: 451–8.

[26] Avise JC, Arnould J, Ball RM, Bermingham E, Lamb T, Neigel JE, Reeb CA, Saunders NC. Intraspecific phylogeography: the mitochondrial bridge between population genetics and systematics. Ann Rev Ecol Syst 1987;18:489–522.

[27] Sperling FAH, Hickey DA. Mitochondrial DNA sequence variation in the spruce budworm species complex (Choristoneura: Lepidoptera). Mol Biol Evol 1994;11:656–65.

[28] Shen J. Digenetic trematodes of fishes from the East China Sea. Five new species of the families Bucephalidae, Fellodistomidae, Zoogonidae and Monorchidae. Stud Mar Sinica 1987; 28:141–9.

[29] Gupta V, Jam N. On some trematodes of the family Bucephalidae Poche, 1907 from marine fishes of Puri, Orissa, India. Pakistan J Zool 1993;25:1–6.

[30] Edgar G. Australian Marine Life: The Plants and Animals of Temperate Waters. Sydney: Reed New Holland; 2000.